Status of the Claims:

Claims 1-76 were originally filed in the case. Claims 1-64 and 74-76 were withdrawn from consideration due to a restriction. Claims 65-73 have been canceled. Claims 77-97 have been added.

Amendments to the Claims:

Newly added claims 77- 97 are believed to more clearly define the transgenic plants that are disclosed in the present application. No new matter has been added. Support is found particularly in Examples 2,3 and 5 as well as throughout the specification.

Rejection of claims 65-73 under 35 U.S.C. §112, First Paragraph

Now canceled claims 65-73 were rejected under 35 U.S.C. §112, First Paragraph, for failing to comply with the written description requirement. The Action describes the invention as directed to transgenic plants whose cells are transformed with any type of exogenous nucleic acid that alters in any way, whether by downregulation or upregulation, expression of any vacuolar pyrophosphatase, including AVP1 or its homologs and likewise including the antiporter NtNHX1 and its homologs. The Action takes the position that the specification does not provide support for the use of any exogenous nucleic acid that downregulates the antiporter genes in the claimed transformed plants, nor is there support for use of homologs of AVP1 or AtNHX1 genes such as from tobacco, bacteria, tomato or corn.

In support, the Action further states that neither a representative number of species falling within the scope of the claimed genus, nor structural features unique to the genus, have been described, citing a written description requirement for cDNAs that was espoused in University of California v. Eli Lilly and Co., 119 F.3d 1559, 1569; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Applicants respectfully note that the Court in *University of California* was not addressing transgenic plants; rather the concern was a claim to novel nucleic acid sequences that had not been specifically identified. It is well settled that a DNA segment must be sequenced in order to be claimed or that a representative number of members of a genus must be shown to have common structural features, usually determined by sequencing and hybridization data (homology assessment).

The examiner is requested to consider the following comments with respect to new claims 77-97, which are believed to be amply supported in the specification, explicitly and in light of what is accepted and recognized in the art.

In the instant case, applicants are not claiming a new nucleic acid sequence. The individual genes are well-known and easily accessed by those of ordinary skill in the art. Applicants used a model yeast system to show how transporters are involved in sodium sequestration (see Figs 3A and 3B). They then showed that the heterologous antiporter gene *AtNHX1* and/or the vacuolar

pyrophosphatase gene could be inserted into plants, using Arabidopsis as a model. They found that either chimeric gene alone or in combination would overexpress its gene product and result in distinct phenotypic alterations. Examples 2, 3 and 5 exemplify results obtained in transgenic Arabidopis plants. Arabidopsis is well-studied and is recognized as a convenient model for identifying altered phenotypes in transformed plants.

Continuing to address the Action's written description concerns, Applicants respectfully direct attention to several places in the specification that are believed to support claims that are not limited to the specific genes used in the model Arabidopsis There is a reasonable expectation that the beneficial characteristics obtained with the genetically engineered Arabidopsis plant would be obtained with other plant species.

Figure 2 and its description on page 5 of the specification illustrates typical alignments of the amino acid sequences of Arabidopsis, human and yeast Na+/H+ antiporter gene-encoded proteins. It should also be noted that the yeast antiporter gene was used to demonstrate overexpression of NHX1 protein in a plant (the model plant). It is therefore to be expected that virtually any antiporter gene, so long as it is an Na+/H+ antiporter gene, will be operable because of the broad species homology, as demonstrated in Fig. 2.

Additional support for the role of antiporters is found in salt stressed plants. Both ATP and pyrophosphate (PPi)-dependent H+ transport are observed in tonoplast vesicles from sunflower seedling roots (see the reference cited at lines 11-12, page 6 in the specification). A particular example cited in the specification on page 6, lines 12-14, is the stimulation of both vacuolar H+-ATPase and a vaculolar Na+/H+ antiporter in the halophyte (plant that grows in salt) *Mesembryanthemum crystallinum*. See also lines 15-19, further supporting applicants' use of Arabidopsis as a model plant and the use of the yeast system in uncovering the mechanisms involved in salt sequestration. Subsequent to the yeast studies, Applicants enginered a non-halophyte plant (Arabidopsis) and demonstrated significant salt tolerance. Transgenic plants that exhibit the several desirable phenotypes have been disclosed.

Rejection of Claims 65-73 under 35 U.S.C. §112, first paragraph

Claims 65-73 stand rejected under 35 U.S.C. §112, first paragraph, as lacking enablement for a transgenic plant that is tolerant of any salt other than sodium chloride. Additionally, the Action takes the position that AVP1 gene homologs from tobacco, bacteria, tomato or corn are not enabled for use in constructing the disclosed transgenic plants.

In view of the new set of claims, applicants submit that any issue of salt tolerance relating to other than sodium chloride is moot; however, applicants would like to point out that they tested their transgenic plants in NaCl and in KCl (page 24, lines 1-3 in third paragraph). While the results were not so spectacular as with NaCl, an increase in salt tolerance was also observed with KCl. As stated on page 14, line 14, in the specification, the vacuolar membrane is implicated in

compartmentation of regulatory Ca^{2+} so that it is reasonable to include salts in addition to NaCl in considering salt tolerance of the disclosed transgenic plants.

Applicants have discussed their rationale for use of a wide range of antiporters and vacuolar pyrophosphatases, and they believe that there is ample written description in the specification to support availability and known homologies of the yeast, human and plant genes. The Action will recognize that it is not necessary to illustrate with examples all equivalents of an invention. Here, when reading the specification, it is clear that the yeast model has shown that substantially any antiporter or vacuolar pyrophosphatase gene that can be expressed in plants will have the function demonstrated; *i.e.,* will express gene products that contribute to additional amounts (compared to normal) of the gene products in the transgenic plant. When these products are "overexpressed", the plant exhibits the described characteristics of salt tolerance.

Enablement for overexpression is explicitly described as operatively linking a 35S promoter double tandem enhancer gene to the antiporter and the vacuolar pyrophosphatase genes. With this information, and in light of the disclosure in the specification, one of skill in the art would have a reasonable expectation that any antiporter or vacuolar pyrophosphatase gene so engineered would have an effect similar to the disclosed chimeric AVP1 and AtNHX1 genes.

Rejection of claims 71-73 under 35 U.S.C. §112, Second Paragraph

Claims 71-73 stand rejected under 35 U.S.C. §112, second paragraph, as indefinite with respect to whether or not the claimed seeds and progeny contain the chimeric gene found in the parent transgenic plant. Applicants new claims state explicitly that the transforming chimeric gene(s) is harbored in the seeds and progeny.

Rejection of Claims 71-73 under 35 U.S.C. §102(a)

Claims 71-73 stand rejected under 35 U.S.C. §102(a) as anticipated by Apse, *et al.* Apse is said to teach seed produced by Arabidopsis transformed with the antiporter AtNHX1. Applicants are uncertain what the specific objection is, but would like to point out that added claims 77-97 include progeny and seed that harbor the same chimera as the parent transgenic plant. Additionally, applicants chimera is an AtNHX1 gene operably linked to a 35S promoter double tandem enhancer, which is different from the Apse construct.

Rejection of Claims 65-66 and 68-73 under 35 §U.S.C. §103(a)

Claims 65-66 and 68-73 stand rejected under 35 §U.S.C. §103(a) as unpatentable over Apse, *et al* in view of Gaxiola, et al and further in view of admitted prior art. Apse is cited as teaching a transgenic Arabidopsis plant that alters expression of the AtNHX1 gene, while Gaxiola is said to teach expression of AVP1 which confers salt tolerance to a yeast mutant and additionally that salt tolerance in yeast occurs only in strains containing a functional Gef1 channel and Nhx1 gene. The Action states that use of the double tandem enhancer of the CaMV 35S promoter in plant transformation constructs was know(n) in the art prior to the invention.

With all due respect, Applicants do not believe that the specification stated that the double tandem enhancer of the CaMV 35S promoter with a chimeric AtNHX1 inserted into a plant was known in the art. The Topfer reference cited in the specification discloses only a vector plasmid harboring the 35S promoter and the PolyA signal of CaMV strain Cabb B-D. Multiple restriction sites were shown for insertion of genes between the 35S promoter and the polyA site. The title of the abstract labels the vector as a plant expression vector for transcriptional and translational fusions. This is not sufficient to render Applicants' construct obvious as merely a "modification of design parameters."

Apse reports that salt stressed wild type Arabidopsis plants produce greater than normal amounts of AtNHX1, the product of the AtNHX1 gene. He also alludes to the salt resistance of transgenic T3 plants that overexpress AtNHX1. As best we can tell, Apse's transgenic plants were prepared by agrobacterium transfection of an AtNHX1 gene under the control of a supernas promoter. The chimeric gene in applicants' transformed plants is under the control of a different promoter.

There is little motivation from the Gaxiola, *et al* publication to use AVP1 gene in combination with the CaMV 35S double tandem enhancer to transform plants, much less to expect the striking results in terms of salt resistance. While the results in yeast may be considered of interest, there is no suggestion or teaching that, without actually transforming a plant, significant salt resistance could be achieved. Moreover, Topfer (the reference listed on page 26, lines 20-22 of the specification) does not teach a double tandem enhancer, as discussed above.

The Apse, *et al.*, Gaxiola, *et al.*, and Topfer reference (cited in the specification) do not render the claimed invention obvious because not one reference provides teaching or motivation for designing applicants' vector and making it work in a model plant.

Rejection of Claims 65-66 and 68-73 under 35 U.S.C. §103(a)

Claims 65-66 and 68-73 have been further rejected under 35 U.S.C. §103(a) as unpatentable over Sheveleva, *et al.*, in view of Barkla, *et al.*, Sarafian, *et al.*, Nass, *et al.* and further in view of "Applicant's admitted prior art."

Sheveleva, *et al.* is cited as disclosing a salt-tolerant transgenic plant where altered expression of IMT1 was correlated with salt stress tolerance. Sheveleva, *et al.* do not teach or suggest that plants transformed with AtNHX1 would have an effect on salt tolerance

Barkla, *et al.* are said to teach that plant Na+/H+ antiporters can transport Na+ into the vacuole because of vacuolar pyrophosphatase and that the antiporter is indicated to affect the ability of plants to tolerate salt. Applicants note that this reference simply investigates the mechanism of Na+ transport at the vacuolar membrane of leaf mesophyll cells of a salt-loving plant (page 550, first full paragraph in Barkla). The authors suggest that there is a relatively close coupling between increases in Na+/H+ antiporter activity and H+-ATPase activity in a salt-tolerant plant (p 555, last paragraph, Col 1). When all was said and done, the authors admitted that "attempts

to identify the ...at the molecular level, will be important for a more complete understanding of the mechanism of salt tolerance in *M. crystallinium*." (p. 555, last paragraph, col 2). This appears to be an invitation to further experiment because these authors are still in the process of investigating the mechanism. Applicants do not believe that mere knowledge of the existence of an antiporter is sufficient to generate expectation that an antiporter CaMV 35S double tandem enhancer construct inserted into a plant would have predictable results.

Sarafian, *et al.* allegedly teach an exogenous nucleic acid encoding Arabidopsis vacuolar pyrophosphatase AVP1, which can alter "expression of vacuolar pyrophosphatase." Applicants observe that Sarafian cloned and sequenced cDNA encoding a pyrophosphate-energized vacuolar membrane proton pump of Arabidopsis, but made no suggestion of the engineering disclosed by applicants to transform plants, particularly plants that are not salt-tolerant, to be salt resistant.

The Action cites Nass, *et al.* as showing that an exogenous nucleic acid encoding the yeast Na+/H+ antiporter NHX1, a homolog of AtNHX1, can alter expression of the gene and that this also confers salt tolerance in yeast. Applicants respectfully disagree with this conclusion. Nass showed that certain mutations in the plasma membrane conferred Na+ tolerance in yeast, but there was nothing to suggest that an exogenous nucleic acid had this effect; *i.e.*, suggestion of a transgenic use. Nass did show homology of the yeast antiporter protein using alignment techniques, but it is not fair to say that this teaches use of an exogenous nucleic acid to produce the effects Nass observed in figuring out what was affecting Na+ transport in mutated yeast plasma membrane H+-ATPase.

The "admission" of applicant's statement that the double tandem enhancer of the 35S promoter of CaM was known, is not correct. The Topfer reference, as discussed, does not disclose the double tandem enhancer.

It is submitted that no combination of the Sheveleva, *et al.*, Barkla, *et al*, Sarafian, *et al.*, Nass, *et al.* and/or Topfer references teaches or suggests the claimed invention. The overall motivation in each of these references appears to be an investigation of mechanisms or identification of particular genes or expressed proteins, certainly without suggestion or motivation to engineer the AtNHX1 and/or AVP1 chimeric genes and insert them into a model plant with the expectation of seeing a significant phenotypic change in a non-halophyte model plant.

Conclusion:

Applicants believe that the Action's concerns have been addressed and that the claims are in condition for allowance. Reconsideration of the application is respectfully requested. The undersigned would appreciate a telephone conference with the Examiner should there be any questions or concerns or should the Examiner have any suggestions.

Respectfully Submitted,

06/21/04
Date

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LISTING OF THE CLAIMS:

1-64 (Withdrawn from consideration)

65-73 (Canceled)

74-76 (Withdrawn from consideration)

NEW CLAIMS 77-97:

77. A transgenic plant having incorporated into its genome a chimeric Na+/H+ antiporter gene or a chimeric vacuolar pyrophosphatase gene each operably linked to a 35S (CaMV) promoter double tandem enhancer gene that causes overexpression of said gene in said plant.

78. The transgenic plant of claim 77 having incorporated into its genome the chimeric Na+/H+ antiporter gene..

79. The transgenic plant of claim 78 wherein the antiporter gene is *At*NHX1 that expresses AtNHX1 protein.

80. The transgenic plant of claim 77 selected from the group consisting of tomato, tobacco, rice, tobacco, sorghum, cucumber, lettuce, turf grass, Arabidopsis and corn.

81. A progeny or seed harboring the chimeric antiporter gene from the transgenic plant of claim 77.

82. The transgenic plant of claim 77 having incorporated into its genome the chimeric vacuolar pyrophosphatase gene.

83. The transgenic plant of claim 82 wherein the vacuolar pyrophosphatase gene is a plant or yeast gene.

84. The transgenic plant of claim 83 wherein the vacuolar pyrophosphatase gene is *AVP1.*

85. A progeny or seed harboring the chimeric vacuolar pyrophosphatase gene of claim 84.

86. A transgenic plant having incorporated into its genome a chimeric vacuolar pyrophosphatase gene and a Na+/H+ exchanger gene, each operably linked to a 35S (CaMV) promoter double tandem enhancer gene that causes overexpression of the vacuolar pyrophosphatase gene and the Na+/H+ exchanger gene in said plant.

87. The transgenic plant of claim 86 wherein the vacuolar pyrophosphatase gene is AVP1.

88. The transgenic plant of claim 86 wherein the Na+/H+ exchanger gene is *At*NHX1.

89. A progeny or seed harboring the vacuolar pyrophosphatase gene and the Na+/H+ exchanger gene of claim 86.

90. A plant cell from the transgenic plant of claim 86 having incorporated into its genome a chimeric vacuolar pyrophosphatase gene and a Na+/H+ exchanger gene, each operably linked to a 35S (CaMV) promoter double tandem enhancer gene that causes overexpression of the vacuolar pyrophosphatase gene and the Na+/H+ exchanger gene in said cell.

91. The transgenic plant of claim 78 wherein the chimeric antiporter gene incorporated into its genome encodes an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3.

92. A progeny or seed which incorporates into its genome the chimeric antiporter gene of the transgenic plant of claim 91.

93. The transgenic plant of claim 1 wherein said antiporter gene overexpresses a protein having the amino sequence of SEQ ID NO. 2.

94. The transgenic plant of claim 91 wherein said antiporter gene overexpresses a protein having the sequence of SEQ ID NO. 3.

95. A transformed plant host cell comprising an antiporter gene that encodes a protein having enhanced proton transporter activity in said cell compared to a counterpart unmodified antiporter gene.

96. A method for producing a genetically transformed plant that exhibits salt tolerance to one or more salts selected from the group consisting of NaCl, KCl and $CaCl_2$, comprising the steps of:

a) inserting into the genome of a plant cell a chimeric gene, which comprises
   i) a DNA sequence encoding an amino acid sequence selected from the group consisting of SEQ ID NO. 1, SEQ ID NO. 2 and SEQ ID NO. 3;
   ii) a 35S CaMV promoter operably linked to the DNA sequence of step i);
   iii) a double tandem enhancer of said promoter
b) obtaining the transformed plant cells; and
c) regenerating a genetically transformed plant from said plant cell wherein said plant exhibits salt tolerance.

97. The genetically transformed plant of claim 96 selected from the group consisting of tomato, tobacco, rice, sorghum, cucumber, lettuce, turf grass, Arabidopsis and corn.